

for detecting the expression of GAPDH as an internal control. Finally, the amplified products were separated on a 1.5% agarose gel.

After the figures, insert pages 1-4 of the Sequence Listing, submitted concurrently herewith.

REMARKS

The specification has been amended pursuant to 37 C.F.R. §1.821 to incorporate the Sequence Listing and sequence identifiers corresponding to the Sequence Listing submitted herewith. No new matter is added by the amendments made herein.

Applicants respectfully request the entry of the amendments made herein into the file of the above-identified application.

Respectfully submitted,

Date: November 14, 2002

Geraldine F. Baldwin 31,232
Geraldine F. Baldwin (Reg. No.)

PENNIE & EDMONDS LLP
1155 Avenue of the Americas
New York, New York 10036-2711
(212) 790-9090

Enclosures

EXHIBIT A

Serial No.: 10/070,350
Filed: February 27, 2002
Attorney Docket No.: 9426-023

MARKED-UP VERSION OF THE SPECIFICATION UNDERLINED TEXT IS ADDED AND [BRACKETED TEXT] IS DELETED

On page 10, please amend the paragraph beginning on line 1 to recite as follows:

Figure 1: OSN Regulatory Region Sequence from -1409 to +904 (SEQ ID NO:1).

On page 10, please amend the paragraph beginning on line 30 to recite as follows:

Figure 11: OSN Regulatory Region Sequence from -522 to +62 (SEQ ID NO:2).

On page 47, please amend the paragraph beginning on line 26 to recite as follows:

All promoter constructs were generated by TOPO TA cloning system (Invitrogen, CA) and subsequently digested using appropriate restriction sites in the polylinker to allow insertion into the vector pGL3-basic (Promega) containing the coding region of the firefly luciferase gene. The OSN 2.3, OSN 1.5, OSN 1.1 and OSN 0.2 promoter constructs were prepared using the primer sequences P1, P2, P3 and P4 as shown in Figure 1. Additional OSN promoter deletion constructs starting at -522 were prepared. These constructs, which also contain a deletion of a spacer bewteen GGA box 1 and 2, contain -522 to +39, -522 to +62 and -522 to +73 of the OSN sequence (See Figures 1 and 11). To create these constructs beginning at -522 of the OSN sequence, PCR was performed utilizing the following primers: 522-N: (5'ACTAGTAGCAGCTTGTCTTGTCT3') (SEQ ID NO:3), spdel-C: (5'CTTCTCCCTGTCTCTGTCTT3') (SEQ ID NO:4); and spdel-N: (5'AAGACAGAGACAGGGGAGAAG3') (SEQ ID NO:5) combined with downstream primers: Intron-C: (5'TACCTCAGTGGCAGGCAGGCAG3') (SEQ ID NO:6), Exon-C: (5'CAGGCAGGCAGGCAGGCAG') (SEQ ID NO:7), and Hafner-C:

(5'GCGCGCTCTCCGGGCAGTCTG3') (SEQ ID NO:8) to construct hON-522I, hON-522E and hONHafner, respectively, and the genomic DNA isolated from DU145 cells as template. All constructs, including the PCR-generated DNA fragments, were confirmed by sequencing.

On page 50, please amend the paragraph beginning on line 8 to recite as follows:

RNA was extracted from cell lines and prostate tumor tissue using RNAzol B reagent (TEL-Test, INC, TX) following the manufacturer's instruction. One microgram of total RNA was used to synthesize the first-strand cDNA using random primers (Perkin Elmer) and MMLV reverse transcriptase (Gibco BRL) in a total volume of 20 μ l. The reaction was performed at 42°C for 1 hour. 2.5 μ l of the RT reaction was adjusted to contain 25 ng of each pair of human osteonectin-specific primers (5'TCCACCACCCTGTTGCTGT3' (SEQ ID NO:9), sense; and 5'CTCCAGGCGCTCTCATT3' (SEQ ID NO:10), antisense) or glyceraldehyde-3-phosphate dehydrogenase (GAPDH)-specific primers (5' ACCACAGTCCATGCCATCA3' (SEQ ID NO:11), sense; 5'TCCACCACCCTGTTGCTGT3' (SEQ ID NO:12), antisense); an additional buffer was added to a total volume of 25 μ l. PCR was then performed for 50 cycles (94°C, 30 second; 55°C, 30 second; and 72°C, 1 min) for detecting the expression of osteonectin and 25 cycles for detecting the expression of GAPDH as an internal control. Finally, the amplified products were separated on a 1.5% agarose gel.